

# **Computational Studies on Effects of Cocaine on Fast-paced Dopamine Neurons**

by

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## **Computational Studies on Effects of Cocaine on Fast-paced Dopamine Neurons**

### **Goals**

The three main goals of this project are:

- to accurately simulate amperometry data showing increase in extracellular dopamine after inhibition of the dopamine transporter,
- to determine the mechanism of action for nomifensin and cocaine based on variations of their effects in a pulse paradigm,
- to show using a fast time scale increment the actual pattern of signaling after inhibition of the dopamine transporter.

### **Introduction**

Dopaminergic neurons are important in the regulation of goal-directed movement, reward, and determination of the value of sensory inputs. Understanding of such phenomena would be enhanced by knowing the detailed kinetics of dopamine in the synapse. Dopamine kinetics are governed by processes that control movement of dopamine into and out of the synapse. The processes include synthesis and metabolism of dopamine in the varicosity, transport into storage vesicles, exocytosis, diffusion in extracellular space, and transport from extracellular space into the cytosol of the varicosity. The contribution of each of these processes to overall dopamine kinetics varies between terminal areas in the brain.

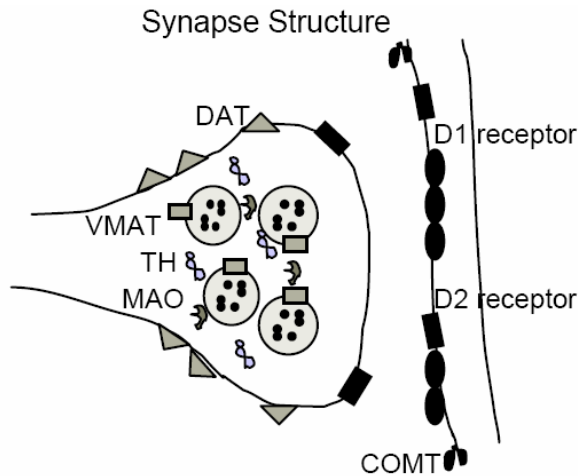


Figure 1. Drawing showing key components and their location in a dopaminergic synapse.

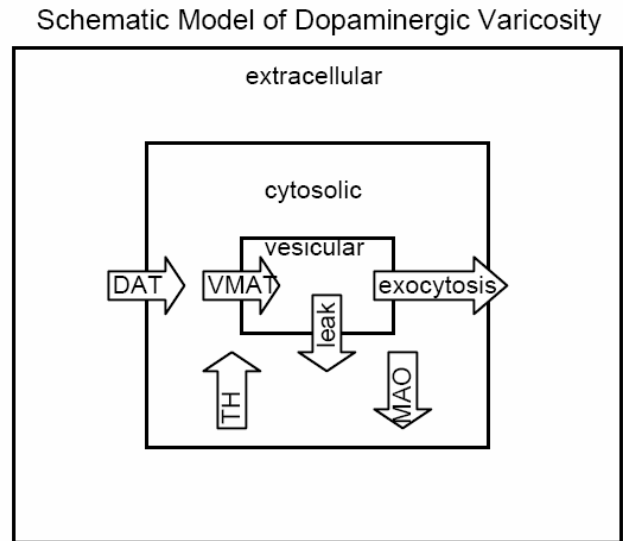
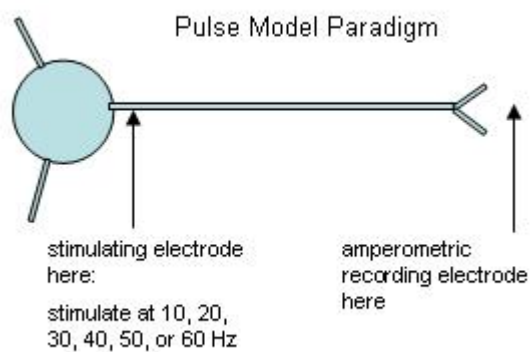


Figure 2. Schematic of dopamine kinetics.

One strategy for understanding a complicated system is to reduce it to simpler components. Such a strategy was employed in this work. We took advantage of an experimental paradigm that stimulated dopaminergic neurons for a maximum duration of two minutes at rates substantially higher than normal brain activity, which has 2-5Hz of stimulation in the neuron. The paradigm is set up in a way so that the stimulating electrode is placed on the axon and the recording electrodes are placed at the axonal terminals. The stimulation paradigm consists of 120 pulses at 10 – 60 Hz with 10 Hz increments. (picture of the axon and the electrodes)



Under the conditions employed in the “pulse” experiments, the processes involving extracellular dopamine are measured while those occurring within the cytosol are not relevant. Thus, in these studies, we are working with three movement mechanism of dopamine. One is dopamine gets released through exocytosis from the pre-synaptic neuron into the synapse. Second is dopamine that is released gets taken up by post synaptic neuron or disappears. The third is extracellular dopamine gets transported back into the pre-synaptic neuron through the dopamine transporter.

For the experiments reported in this paper, a computer simulation model of the pulse experiment was used. Values for each of the parameters controlling dopamine location and movement were input into the model and the model output was compared to experimental data gathered from Garriss and Wightman.<sup>(1)</sup> Values were varied until the output results closely matched published experimental data. Then, the impact of various mechanisms of inhibiting dopamine transport was evaluated in the computer simulation model. Four different mechanisms by which the transporter can be inhibited were examined. One is a competitive inhibition at the binding site for dopamine, second is a non-competitive inhibition that slows the rate constant for dopamine interacting with the transporter, the third is an interaction that inactivates the transporter, and a the fourth a slowing of the rate that the transporter moves dopamine across the membrane. The results of these four mechanisms of inhibiting the transporter were then compared to the pattern of published experimental results using cocaine and nomifensin in the “pulse” experimental model. Finally, a set of parameters that gave a simulation output that was a good match to effects of cocaine was used to predict the effects of cocaine on actual, “real time” amounts of dopamine in the extracellular space.

## Methods

The modeling process, developed as a spreadsheet using Microsoft Excel, is accomplished by the following strategy:

1. A value is assigned for each process that affects dopamine in the “pulse” model. The processes included were
  - a. volume of extracellular space associated with an individual varicosity
  - b. number of dopamine transporters located on an individual varicosity
  - c. affinity of the dopamine transporter for dopamine
  - d. time increment required for a transporter to move one molecule of dopamine across the membrane
  - e. number of dopamine molecules in each exocytotic event
  - f. rate constant for dopamine removal from extracellular space by processes other than the dopamine transporter.
2. An initial value is assigned for dopamine in each of the three compartments.
3. The model proceeds through sequential “time” steps. At each step, the model:
  - a. calculates how much dopamine is moved between compartments by exocytosis, the dopamine transporter, and other extracellular processes
  - b. computes a new value for dopamine in extracellular space by adding to the current value of dopamine any dopamine exocytosed and subtracting any dopamine transported out of extracellular space.
4. The calculations of step #3 are repeated until the desired amount of time is simulated.

5. The amounts of dopamine in the extracellular space during the course of the simulation are compared to experimentally measured values.
6. Parameter values are adjusted, and steps #3-#5 are repeated until a good match between model output and experimentally measured values is achieved.

The time interval represented by each iteration in the program was set at 0.00167 seconds.

The amount of dopamine moved by a transporter was calculated as the product of the dopamine in the extracellular compartment times the affinity constant of the transporter for dopamine times the concentration of dopamine transporters. A check was then done to verify that the amount of dopamine to be moved did not exceed the number of transporters. When the calculation showed more dopamine to be moved than transporters, then amount of dopamine movement was set equal to the number of transporters.

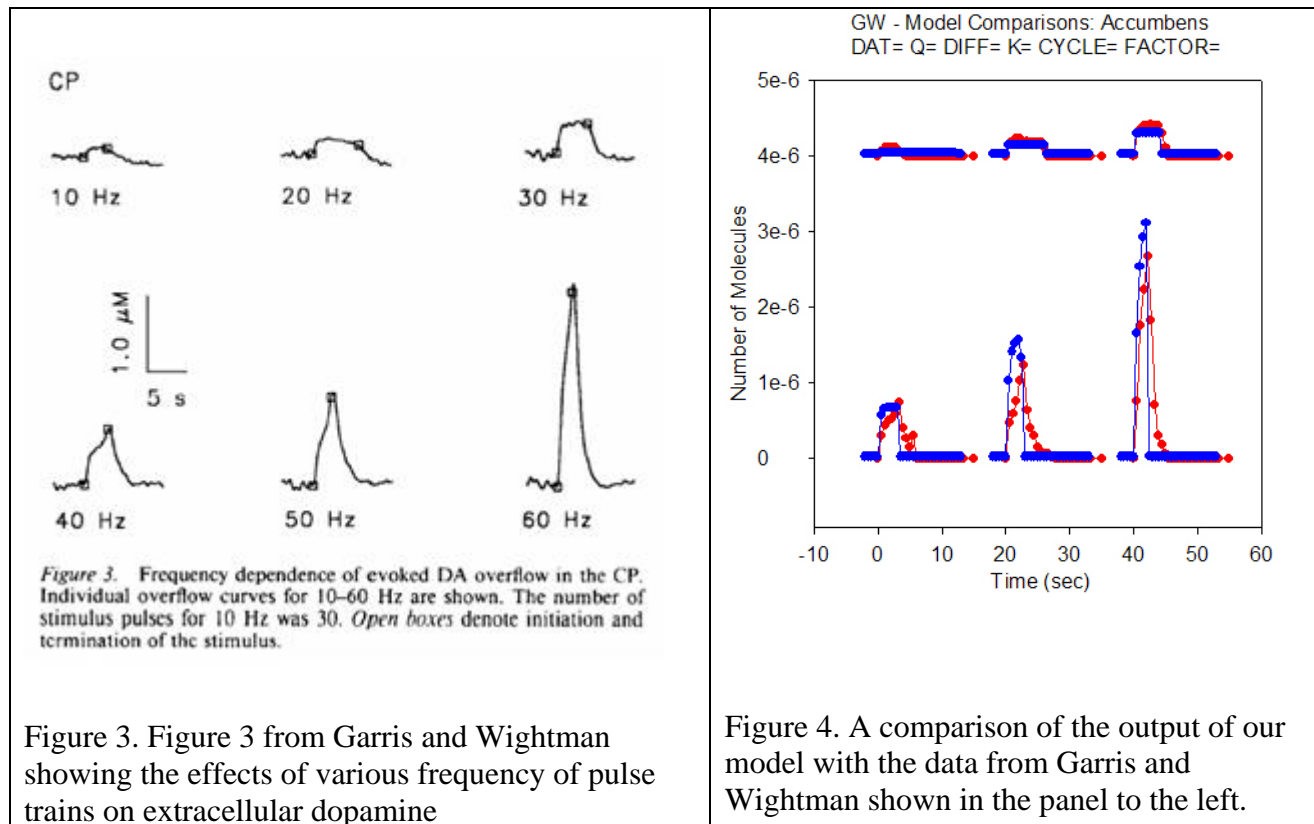
Dr. Wallace's past research had established baseline parameter values for the pulse simulation model for striatum. Some of the initial starting values were based on neurochemical data. For example, binding data coupled with quantitative electron microscopy data were used to establish the number of dopamine transporters on an individual varicosity and the volume of the extracellular space associated with an individual varicosity. The affinity of the dopamine transporter for dopamine was set such that the half-time for dopamine disappearance from extracellular space was close to the 50 msec value from published data. The value for non-transporter-mediated removal from extracellular space was set at ~3% of the transporter-mediated movement based on rate of dopamine disappearance from extracellular space in transgenic animals lacking the dopamine transporter or in animals in which the transporter is pharmacologically blocked. Finally, biological and biochemical data suggest that the number of

dopamine molecules involved in each exocytotic event is less than 2000. These initial values were then refined by trial and error to obtain a set of values that was a good match to experimental data.

For my project, I investigated the effects of four different mechanisms of inhibiting the dopamine transporter. A non-competitive mechanism that inactivates the transporter was modeled as a decrease in the number of dopamine transporters associated with an individual varicosity. A non-competitive mechanism that slows the rate constant for dopamine interacting with the transporter was modeled as a decrease in the affinity constant of the dopamine transporter for dopamine. The slowing of the rate that the transporter moves dopamine across the membrane was modeled as an increase in time increment needed for a single transporter to move one dopamine molecule across the membrane. Finally, a competitive interaction was modeled by adding parameters for competing molecules to the program.

## **Results**

The first set of data validates the computer simulation model that was used. The results show that the model output is a close match to the data published by Garriss and Wightman.<sup>(1)</sup>



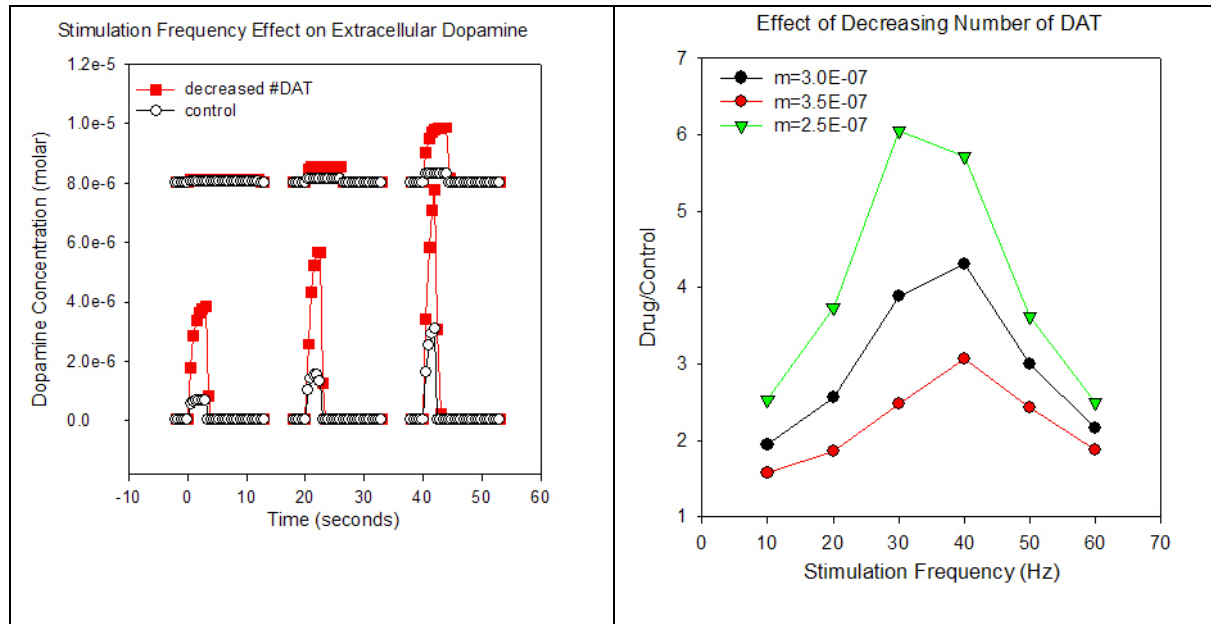
The program parameter values used to achieve this match to the experimental data were: 2,850 dopamine transporters on an individual varicosity,  $9.5\text{E-}15$  liters of extracellular space associated with an individual varicosity, an affinity value of the dopamine transporter for dopamine of  $\text{DAT}_k = 3.5\text{E-}33$ , 340 dopamine molecules involved in each exocytotic event, a probability constant of dopamine being removed from extracellular space by some process other than the dopamine transporters of  $2\text{E-}17$ , and a time for the transporter to move a single dopamine molecule across the membrane of 0.17 seconds.

*Part 1: TO SHOW THE EFFECT OF VARIOUS MECHANISM OF INHIBITION OF DOPAMINE TRANSPORTER*



We used the pulse program to evaluate the effects of various mechanisms of inhibition of the dopamine transporter on the pattern of extracellular dopamine when the pulse frequency was varied.

Effects of decreasing number of dopamine transporters. We first looked at the effect of decreasing the number of dopamine transporters (non-competitive inhibition decreasing  $V_{max}$ ). In this experiment, the DAT-density values were varied and decreased. The graph on the left shows the dopamine concentration over time with increasing stimulation frequency from 10-60 Hz. The panel on the left shows the output data for control and for the situation where the number of transporters is half of the control value for each of the pulse frequencies. The panel on the right shows data where the peak extracellular dopamine occurring as a result of a stimulus of pulses in the presence of reduced dopamine transporter is divided by the peak value for the control situation. This shows that maximum percent increase in dopamine occurs when the pulse frequency is in the 30-40 Hz range. For the situation where the number of dopamine transporters is half of the control (green line), the graph shows that at lower stimulation frequencies, the extracellular dopamine over the control is approx. 2.5. The extracellular dopamine is 5-6 times more than the control when the frequency is 30-40Hz shown by the peak in the green line. Then the ratio of the dopamine concentration compared to control decreases to 2.5 when the frequency reaches 60Hz.



To understand this pattern of results, one needs to examine the factors that control amount of dopamine in extracellular space. The extracellular dopamine level is a function of the rate of dopamine secretion into the extracellular space (number of dopamine released per exocytotic event times the frequency of those events) minus the clearance rate of the dopamine. Clearance rate is equal to the affinity of the dopamine transporter for dopamine times the number of dopamine transporters available times the rate at which dopamine can be moved across the membrane (the inverse of the cycle time). Using these principles, the pattern of results can be explained by the following:

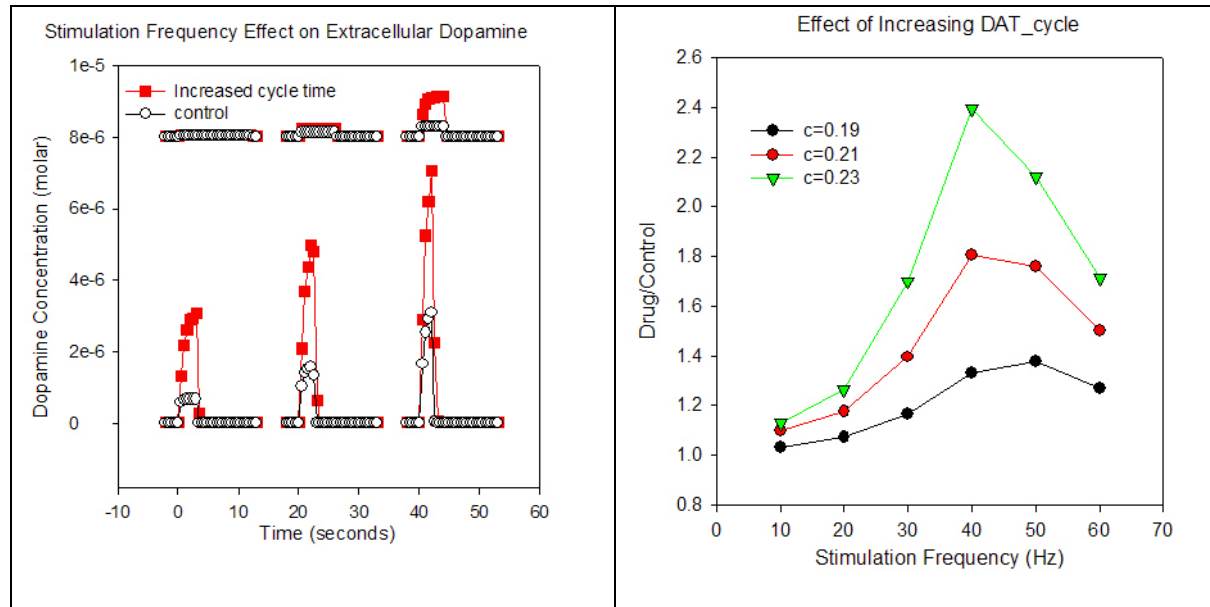
- If the extracellular DA < # DAT, then the decrease in #DAT has very minimal effect on the extracellular dopamine level because the number of excess transporters has simply been decreased.
- If the extracellular DA exterior = #DAT, then the decrease in #DAT has substantial effect on the extracellular dopamine exterior level because the rate limiting factor in removal of dopamine from extracellular space has been decreased.

- If the DA exterior is  $\gg$  #DAT, then the rate limiting process in removal of dopamine from extracellular space is the time it takes for a transporter to move each dopamine molecule. Therefore, the change in number of transporters is not a change in the rate limiting step for dopamine removal from extracellular space, and the extracellular dopamine concentration is only minimally changed.

Thus, at low stimulation frequencies, the affinity of the dopamine transporter for dopamine is the rate limiting step in dopamine clearance. For mid-range stimulation frequencies, the rate limiting factor is a mixture of transporter affinity, number of transporters, and rate of transport. At high stimulation frequencies, the cycle time is the rate limiting step. So, in this experiment, one can see that at both low and high stimulation frequency, the dopamine concentration for the both control and half the control level of DAT will have minimal effect on the extracellular dopamine level; thus, the ratio of experimental to control values will be low.

Effects of increasing the time increment needed to move a single dopamine molecule across the membrane. In this experiment, the DAT-cycle time values were varied and increased. The graph on the left shows the extracellular dopamine concentration over time with increasing stimulation frequency from 10-60 Hz. This graph shows data from the control value of 0.17 seconds and an increased value of 0.23sec cycle time. The graph on the right shows data where the peak extracellular dopamine occurring as a result of a stimulus of pulses in the presence of slowed dopamine transporter is divided by the peak value for the control situation. The green line represents the ratio of the extracellular dopamine over control with the cycle time lengthened by 35% compared to control. The graph shows that at lower stimulation frequencies, the extracellular dopamine over the control is approximately the same with the ratio of 1. The dopamine exterior is 2.4 times higher than the control when the frequency is about 40Hz as

shown by the peak in the green line. Then the ratio of the dopamine concentration compared to control decreases to 1.7 when the frequency reaches 60Hz at 0.23 cycle time to that of control which is 0.17.

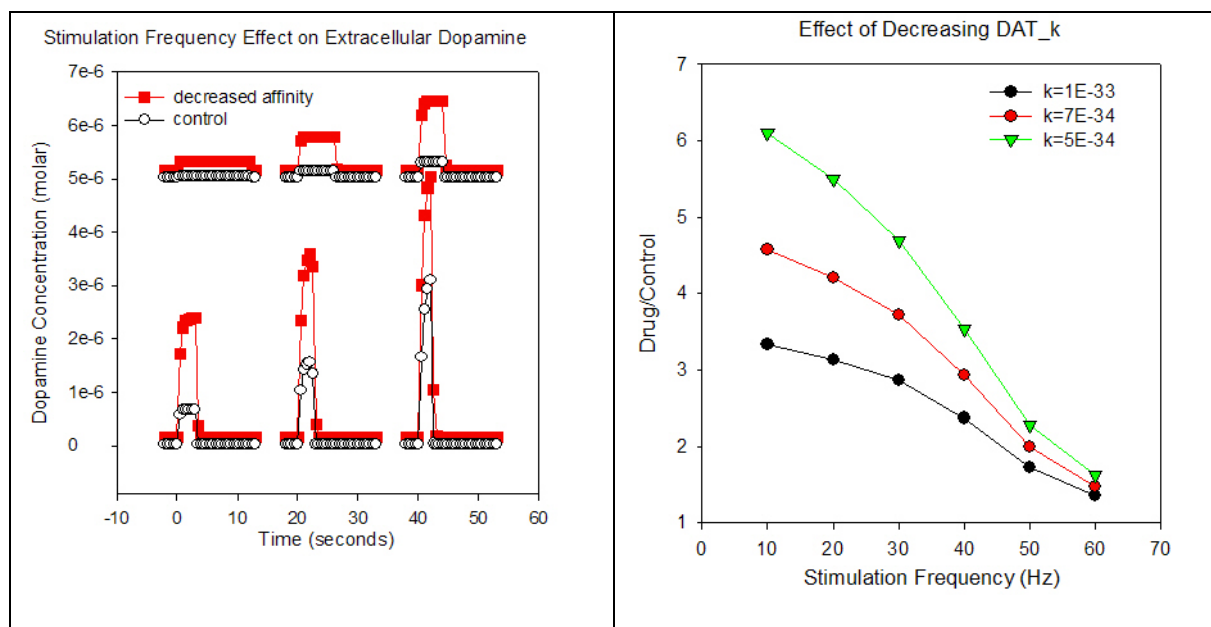


This pattern of results can be explained by the following:

- If the extracellular DA  $< \#$  DAT, then the decrease in rate of transport has very minimal effect on the extracellular dopamine level because the excess transporter capacity has simply been decreased.
- If the extracellular DA exterior =  $\#$  DAT, then the decrease in  $\#$  DAT has substantial effect on the extracellular dopamine exterior level because the rate limiting factor in removal of dopamine from extracellular space has been slowed.
- If the DA exterior is  $\gg \#$  DAT, then the rate limiting process in removal of dopamine from extracellular space is the time it takes for a transporter to move each dopamine molecule. Therefore, slowing of the rate at which each transporter is working is not a

change in the rate limiting step for dopamine removal from extracellular space, and the extracellular dopamine concentration is only minimally changed.

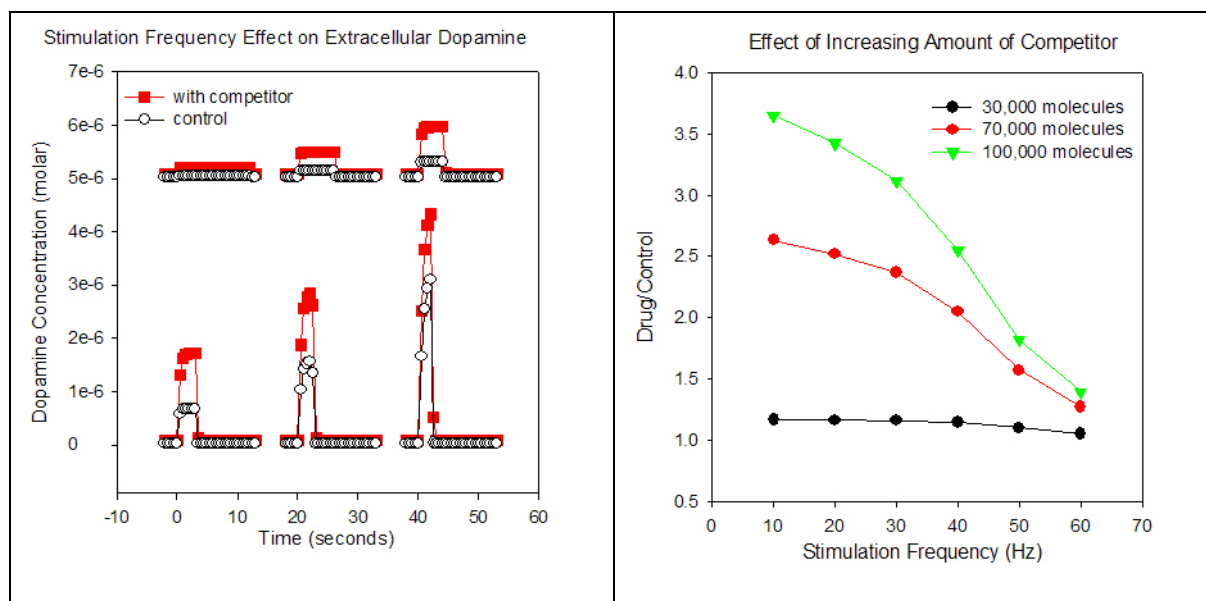
Effects of decreasing the dopamine transporter affinity for dopamine. In this experiment, the DAT affinity values were varied and decreased. The graph on the left shows the extracellular dopamine concentration over time with increasing stimulation frequency from 10-60 Hz. This graph shows data from the control value of  $k=5E-33$  and the experimental value of  $k=5E-34$  (a 90% decrease in affinity). The graph on the right shows data where the peak extracellular dopamine occurring as a result of a stimulus of pulses in the presence of slowed dopamine transporter is divided by the peak value for the control situation. The green line shows the results with a 90% decrease in affinity. The graph shows that at lower stimulation frequencies, the extracellular dopamine over the control is approximately 6 times more than for the control for the green line (90% decrease in affinity). The ratio of experimental to control extracellular dopamine then decreased as the stimulation frequencies increased, with the ratio being only 1.5 when the frequency reaches 60Hz (for the 90% decreased affinity data).



This pattern of results can be explained by the following:

- If the extracellular DA  $< \# \text{DAT}$ , then the decrease in affinity is decreasing the rate limiting step for dopamine clearance, and the amount of extracellular dopamine will be substantially impacted.
- If the extracellular DA exterior =  $\# \text{DAT}$ , then the decrease in  $\# \text{DAT}$  has a moderate effect on the extracellular dopamine exterior level because affinity is only one of several rates limiting factor in removal of dopamine from extracellular space.
- If the DA exterior is  $\gg \# \text{DAT}$ , then the rate limiting process in removal of dopamine from extracellular space is the time it takes for a transporter to move each dopamine molecule. Therefore, a change in affinity is not a change in the rate limiting step for dopamine removal from extracellular space, and the extracellular dopamine concentration is only minimally changed.

Effects of a competitive drug. In this experiment, the model tested the effects of a drug that competes with dopamine for binding to the dopamine transporter. The graph on the left shows the extracellular dopamine concentration over time with increasing stimulation frequency from 10-60 Hz. This graph shows data from the control value compared to the situation with 70,000 non-dopamine molecules that have the same affinity as dopamine for the dopamine transporter. The graph on the right shows data where the peak extracellular dopamine occurring as a result of a stimulus of pulses in the presence of a competitor for binding to the dopamine transporter is divided by the peak value for the control situation. This graph shows the same pattern of effects as observed in the previous study examining the effects of decreasing affinity of the dopamine transporter for dopamine. Thus, the effects were largest at low stimulation frequencies and decreased as the stimulation frequencies were higher.



Since a competitive inhibitor is mechanistically the same as apparent decrease in affinity, the explanation provided for the effects of decreasing affinity applies to this situation with a competitive inhibitor.

*Part 2: THE MECHANISM OF ACTION FOR NOMIFENSIN AND COCAINE ARE TO BE DETERMINED BASED ON VARIATIONS OF THEIR EFFECTS IN A PULSE PARADIGM.*

In this experiment, we wanted to figure out the mechanism with which cocaine and nomifensin work in the brain. The following graphs are taken from papers reporting the effects of cocaine and nomifensin in the “pulse” experimental paradigm.

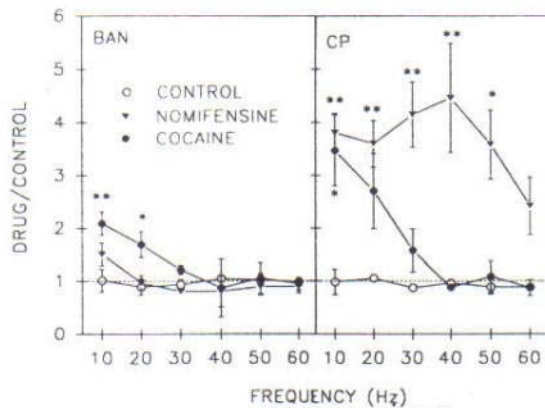
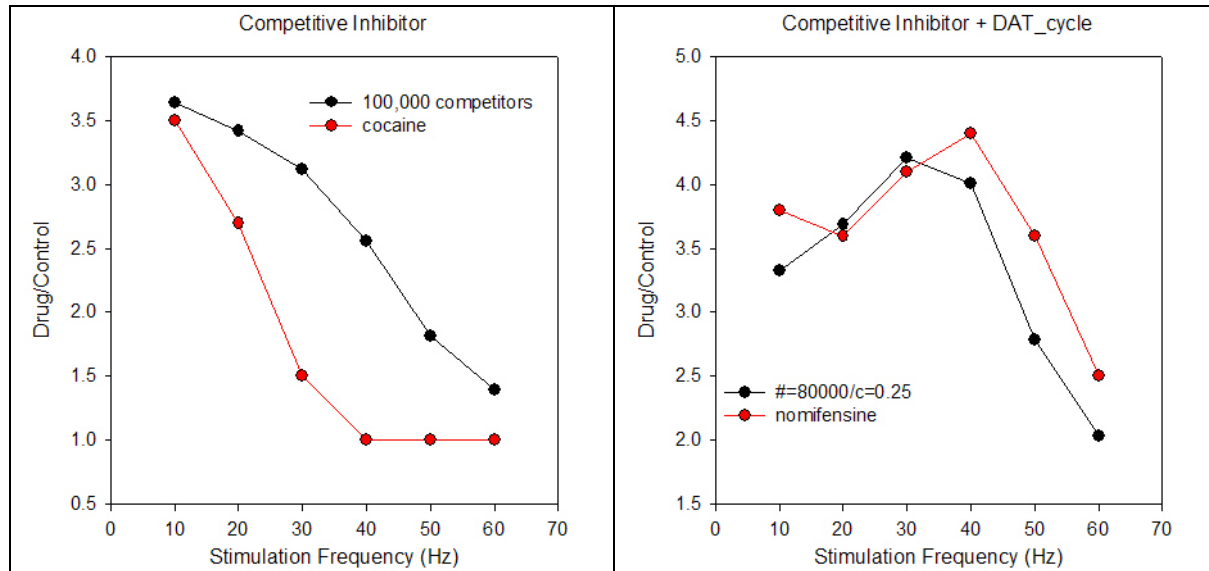


Fig. 2. Frequency dependence of the effects of nomifensine and cocaine on evoked dopamine efflux. Dashed lines indicate a ratio of drug/control of 1. Data are the mean  $\pm$  S.E.M. (at least three determinations in 4 to 5 rats).

This graph shows the striatum and nucleus accumbens regions responses towards two different drugs shown, cocaine and nomifensine. This is the literature data from the Garris and Wightman.

A comparison of the results for cocaine with the four different mechanisms explored in ‘Part 1’ of this project shows that the cocaine pattern matches the pattern observed for a competitive inhibitor. However, the nomifensin data did not match the pattern associated with any of the four mechanisms we explored. We then considered that nomifensin might work via more than one mechanism. We ran computer simulations involving two mechanisms and found a set of conditions that matched the experimentally observed nomifensin data. The optimum results were obtained using a competitive model coupled with a 47% increase in time required for the dopamine transporter to move one dopamine molecule across the membrane. Data comparing cocaine and nomifensin simulations to experimental data are shown in the graph below.

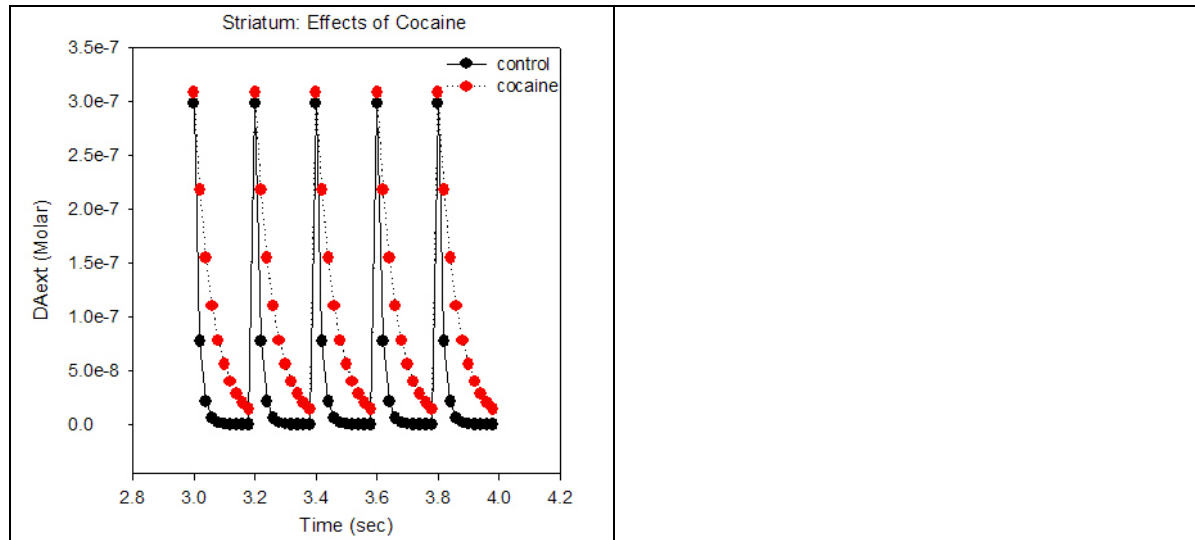




*Part 3: TO SHOE THE ACTUAL PATTERN OF SIGNALING AFTER INHIBITION OF THE DOPAMINE TRANSPORTER.*

Measurements of extracellular dopamine are done using either microdialysis or amperometric techniques. Microdialysis has a time resolution of several minutes. Amperometric techniques has a time resolution of about half a second. Dopamine signaling in the striatum region of the brain occurs on a sub-second time scale. Dopamine neurons are firing approximately 5 times per second, meaning that an exocytotic event followed by dopamine retrieval from the extracellular space followed by the system being reset for the next cycle occurs every 0.2 seconds. Neither the dialysis technique nor the amperometric technique can show extracellular dopamine levels on this time scale. Rather, these techniques are providing average values over a period of time. Using our models, we are able to predict what the extracellular dopamine levels should be throughout a signaling cycle. We ran such computer simulation models for both a control situation and the situation in the presence of cocaine, choosing

parameters for the cocaine that caused a 3-4 fold increase in extracellular dopamine at 10 Hz in the “pulse” computer models. The results are shown in the following graph.



In this simulation, extracellular dopamine peaks immediately following an exocytotic event. The dopamine is then retrieved from the extracellular space by the dopamine transporter. In the control situation (black line), the extracellular dopamine rapidly returns to close to zero following each exocytotic event. In the presence of cocaine, the peak value for extracellular dopamine is only marginally changed (red line). However, the rate at which the dopamine is retrieved from the extracellular space is markedly delayed, barely reaching close to zero in time for the next exocytotic event. The area between the curves tells us the overall effect of cocaine. The average increase in extracellular dopamine in this experiment is 300%. The graphs demonstrate that this is all accounted for by more sustained concentrations of dopamine rather than increased peak concentrations. If one assumes that receptor occupancy is proportionally to extracellular dopamine at concentrations found in the synapse, this suggests that the behavioral effects of cocaine result from an increased duration of dopamine receptor occupancy by dopamine molecules rather than an increased number of occupied receptors.

## Conclusions

This project has investigated the effects of inhibition of the dopamine transporter on extracellular dopamine when dopaminergic neurons are electrically paced at rates substantially higher than those occurring in the brain. The project reported here has used computer simulation models of the data reported from such experiments to learn more about the dopamine transporter. The advantage of using this model is that only the extracellular parameters of dopamine kinetics need to be included, eliminated the need for a more complex model that also includes cytosolic kinetic parameters. The first part of the study evaluated four mechanisms by which the dopamine transporter can be inhibited. One is a competitive inhibition at the binding site for dopamine, second is a non-competitive inhibition that slows the rate constant for dopamine interacting with the transporter, the third is an interaction that inactivates the transporter, and a the fourth a slowing of the rate that the transporter moves dopamine across the membrane. The results show that the competitive inhibition and decreasing the affinity of the dopamine transporter for dopamine models produce the same results. The impact of the inhibitor is substantial at low neuron stimulation frequencies and minimal at high neuron stimulation frequencies. While slowing the rate of transport and decreasing the number of transporters did not give identical results, the pattern of effects was the same for these two mechanisms. The impact of inhibition was minimal at low dopamine neuron stimulation frequencies, substantial at intermediate neuron stimulation frequencies, and minimal at high neuron stimulation frequencies. Published patterns of effects of cocaine in the “pulse” experimental pattern matched our simulations for competitive inhibition. Published patterns of effects of nomifensin in the “pulse” experimental pattern did not match any of our simulations. However, nomifensin data could be matched using a combination of competitive inhibition with a slowing of the transporter rate. The parameters that gave the best

match of cocaine data in our simulations as compared to experimental data were used to estimate the impact of cocaine on extracellular dopamine under baseline conditions. These results demonstrate that the average level of extracellular dopamine over time is increased as a result of longer duration of low levels of dopamine in the synapse rather than increases in peak levels of dopamine. If one assumes that receptor occupancy is proportionally to extracellular dopamine at concentrations found in the synapse, this suggests that the behavioral effects of cocaine result from an increased duration of dopamine receptor occupancy by dopamine molecules rather than an increased number of occupied receptors.

## References:

1. Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: an in vivo voltammetric study, *Journal of Neuroscience* 14:442-450:1994